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Catalytic Activities of Alkaline Phosphatase and N-Acetyl-β-*D*-Glucosaminidase in Human Cortical Nephron Segments: Heterogeneous Changes in Acute Renal Failure and Acute Rejection Following Kidney Allotransplantation

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Dedicated to Professor Dr. med. Adalbert Bohle, Tübingen, on the occasion of his 65th birthday

Summary: The catalytic activities of alkaline phosphatase and N-acetyl-β-*D*-glucosaminidase, constituents of luminal brush-border membranes and lysosomes of kidney tubular cells, were measured in human kidney allografts in the maintenance and recovery phases of acute renal failure and in acute rejection crisis. The enzyme activities were fluorometrically determined in single microdissected cortical nephron segments of biopsies from 4 kidney allografts taken intraoperatively and postoperatively at different periods, which exhibited either good function or dysfunction. For comparison, the unaffected part of a human kidney nephrectomized due to hypernephroma as well as a biopsy of a morphologically normal human kidney were examined.

Both enzymes displayed highest activities in the proximal part of the human nephron. In some intraoperative and postoperative biopsies with acute renal failure, alkaline phosphatase activity was reduced in proximal tubules, predominantly in the straight portion. This reduction could not be correlated with function. In acute rejection, very low alkaline phosphatase activities were uniformly found in proximal convoluted and straight tubules. Furthermore, intraoperative biopsies and biopsies of the functioning allograft have only approximately 50% of normal N-acetyl-β-*D*-glucosaminidase activity in proximal convoluted tubules, but generally normal values in the straight portion. However, in acute renal failure, this enzyme activity was several-fold enhanced along the whole nephron, when compared with intraoperative values. In acute rejection, N-acetyl-β-*D*-glucosaminidase activity was slightly reduced in proximal convoluted tubules, when compared with biopsies showing good function.

It is suggested that the decrease of proximal tubular enzyme activities is the consequence of increased enzymuria and inadequate enzyme regeneration. On the other hand, the overshoot of N-acetyl-β-*D*-glucosaminidase activity in the maintenance phase of acute renal failure appears to indicate increased degradative capacity, associated with cellular regeneration along the whole nephron.

Katalytische Aktivitäten von alkalischer Phosphatase und N-Acetyl-β-D-glucosaminidase in menschlichen corticalen Nephronsegmenten: Heterogene Veränderungen bei akutem Nierenversagen und akuter Abstoßung nach Nierentransplantation

Zusammenfassung: Die katalytischen Aktivitäten von alkalischer Phosphatase und N-Acetyl-β-*D*-glucosaminidase, Bestandteilen von luminalen Bürstensaum-Membranen und Lysosomen der Nierentubulus-Zellen, wurden in menschlichen Nierentransplantaten in Spät- und Erholungsphase akuten Nierenversagens und bei akuter Abstoßungskrise gemessen. Die Enzymaktivitäten wurden fluorometrisch in einzelnen, mikrodissezierten corticalen Nephronsegmenten von Biopsien aus 4 Nierentransplantaten bestimmt, die intraoperativ und postoperativ zu verschiedenen Zeiten bei guter oder schlechter Funktion gewonnen wurden. Zum Vergleich wurden der normale Teil einer menschlichen Niere mit Hypernephrom und die Biopsie einer morphologisch normalen menschlichen Niere untersucht.

Beide Enzyme haben ihre höchsten Aktivitäten im proximalen Teil des menschlichen Nephrons. In einigen intraoperativen und postoperativen Biopsien bei akutem Nierenversagen war die Aktivität der alkalischen Phosphatase in den proximalen Tubuli reduziert, und zwar bevorzugt in den geraden Abschnitten. Diese Reduktion verlief nicht parallel dem klinischen Funktionszustand. Am stärksten und einheitlich in den gewundenen und geraden proximalen Tubuli war diese Enzymaktivität reduziert in der akuten Abstoßungsreaktion. In den intraoperativen Biopsien und in den Biopsien des funktionsfähigen Transplantats war die katalytische Aktivität der N-Acetyl-β-*D*-glucosaminidase in den proximalen Konvoluten etwa auf die Hälfte der normalen reduziert, während sie in den geraden proximalen Tubuli im allgemeinen auf Normalniveau blieb. Dagegen wurde im akuten Nierenversagen eine gegenüber den intraoperativen Werten mehrfach erhöhte Aktivität in allen Nephronsegmenten gefunden. Bei der akuten Abstoßungsreaktion war diese Enzymaktivität in den proximalen Konvoluten leicht reduziert gegenüber den Biopsien im funktionsfähigen Zustand.

Die Abnahme der Enzymaktivitäten in den proximalen Tubuli wird als das Ergebnis vermehrter Enzymurie und nicht angepaßter Enzym-Neusynthese angesehen. Andererseits wird die Erhöhung der Aktivität der N-Acetyl-β-*D*-glucosaminidase in der Spätphase akuten Nierenversagens als Zeichen von vermehrter Abbaukapazität bei der Zellregeneration im ganzen Nephron gedeutet.

Introduction

Alkaline phosphatase (orthophosphoric-monoester phosphohydrolase, alkaline optimum, EC 3.1.3.1) and N-acetyl-β-*D*-glucosaminidase (2-acetamido-2-deoxy-β-*D*-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30) are kidney tubular enzymes which are known to be excreted into urine. Enzymuria increases, when renal function is impaired (1, 2, 3), indicating renal damage. However, the renal processes involved in enzymuria are not yet understood.

In the kidney, alkaline phosphatase is essentially found within the proximal part of the nephron (4), constituting an integral part of luminal brush-border membranes (2). The function of this enzyme is not yet known (5, 6). N-acetyl-β-*D*-glucosaminidase is distributed along the whole nephron with the highest activity in proximal tubules (7). It is localized within the lysosomes of tubular cells (8) and contributes to the intracellular degradation of carbohydrate-containing macromolecules (9).

Brush-border membranes and lysosomes are cellular compartments which undergo alterations in experimentally induced acute renal failure (10). In human kidneys, normal distribution pattern and pathological changes of these organelles along the nephron have not yet been quantified. Morphological analysis of human kidney biopsies taken in acute renal failure revealed a reduced luminal surface area in proximal tubules (11, 12) and an increased number of vacuolar structures in proximal and distal tubules (11). In addition, *Grégoire & Gepts* (13) observed reduced activity of microvillar alkaline phosphatase and unchanged activity of lysosomal acid phosphatase in proximal convoluted tubules of human renal homotransplants, which had been removed due to chronic insufficiency.

The present study is based on recent observations on marker enzymes of cellular compartments along the human nephron. These earlier findings showed that the enzymes of basolateral interdigitations and mitochondrial inner membranes, Na^+-K^+-ATPase and succinate dehydrogenase were selectively reduced in distal tubules of biopsies taken from human kidney allografts in the maintenance phase of acute renal failure (14). These enzyme activities were found to be unchanged in proximal tubules, but in this part of the nephron, marked activity changes were found

with respect to lysosomal N-acetyl-β-*D*-glucosaminidase (15). This finding suggests that in these biopsies, proximal tubules had also undergone alterations, but of a different kind. In order to substantiate this, the measurement of N-acetyl-β-*D*-glucosaminidase activity was performed in all available cortical nephron segments, in addition to the determination of microvillar alkaline phosphatase activity. The same biopsies as in the earlier study were examined, in addition to a biopsy taken in acute rejection crisis (15). The study of intranephronal distribution of both enzyme activities in correlation with renal function should give further insight into the nature and location of cellular events and of enzymuria associated with impaired renal function in humans.

Materials and Methods

Chemicals

Chemicals were of analytical grade from E. Merck AG, D-6100 Darmstadt (buffer substances, liquid paraffin), from Serva Feinbiochemica GmbH & Co., D-6900 Heidelberg (4-methylumbelliferone, 4-methylumbelliferyl-N-acetyl-β-*D*-glucosaminide, bovine serum albumin), and from Sigma Chemical Co., St. Louis, U.S.A., Mo 63178 (4-methylumbelliferyl phosphate).

Tissue preparation

Biopsies were taken from 4 human kidney allografts (A, B, C, D) which had been perfused with Euro-*Collins* solution and preserved on ice for up to 26–32 hours prior to transplantation. Immediately after transplantation, allografts A, B, and C were anuric and did not show any symptoms of cellular or humoral rejection at the time of biopsy (A_2, B_2, C_2), while allograft D displayed diuresis developing into normuria (D_2, D_3) and then into acute rejection crisis (D_4) manifested by decreased creatinine clearance (15) and interstitial round-cell infiltration. Diuretics were not given. Immunosuppressive treatment was conventional. In addition, biopsies of these allografts had been obtained intraoperatively about 30 min after anastomosis (A_1, B_1, C_1, D_1). In comparison with these transplant biopsies, the unaffected part of a human kidney nephrectomized due to hypernephroma grade I (E) and a morphologically normal biopsy taken due to microscopic haematuria (F) were examined.

Tissue samples were prepared for microdissection (16) by immediate shock-freezing in liquid nitrogen and freeze-cutting into 16 µm thick serial sections (Dittes-Duspiva, D-6900 Heidelberg). The first of three consecutive sections was stained with periodic acid/*Schiff*'s base reagent, the second lyophilized, and the third was stained for succinate dehydrogenase activity, as described previously (17). Dry weight of microdissected cortical nephron segments was in the range of 1–40 ng.

Determination of enzyme catalytic activities

Enzyme assays for single nephron segments (4, 7) were modified with respect to incubation conditions and the use of the oil-well technique (16). Sodium carbonate buffer, 0.1 mol/l, pH 10.0, containing 0.2 g/l bovine serum albumin, or sodium citrate buffer, 0.1 mol/l, pH 5.0, containing 0.2 g/l bovine serum albumin (630 nl), was placed into the wells of a teflon rack, loaded with a tissue specimen, and then covered with 2 droplets of liquid paraffin. After cooling to 4 °C, 630 nl of enzyme reagent were added. After incubation for 32 min at 37 °C (2 min for temperature equilibration), the rack was cooled again to 4 °C. Incubation solution (950 nl) was pipetted into 200 µl of 0.5 mol/l glycine buffer, 0.5 mol/l NaCl, pH 10.4. Reagent blanks and 4-methylumbelliferone standards (2–20 µmol/l) were treated in the same manner. Tissue blanks were obtained by omitting incubation for 32 min at 37 °C. Fluorescence measurements were performed at 365/450 nm with a *Farrand* Mark I fluorometer.

Enzyme reagents were composed of: 2 mmol/l 4-methylumbelliferyl phosphate and 8 mmol/l $MgSO_4$ in 0.1 mol/l sodium carbonate buffer, pH 10.0, 0.2 g/l bovine serum albumin, for alkaline phosphatase, the substrate concentration being around saturation; 1.6 mmol/l 4-methylumbelliferyl-N-acetyl-β-*D*-glucosaminide in 0.1 mol/l sodium citrate buffer, pH 5.0, 0.2 g/l bovine serum albumin, for N-acetyl-β-*D*-glucosaminidase, the substrate concentration being in the range of half-saturation.

Specific enzyme activities were expressed in µmol · min^{-1} 4-methylumbelliferone formed per g tissue dry weight. Since tissue blanks of both enzyme assays were at the detection limits for glomeruli and distal nephron segments and were below 6% for proximal tubular values, they were neglected. Differences between the means of enzyme catalytic activities were tested for statistical significance with *Student*'s t test for unrelated samples.

Results

Control biopsies

In addition to the earlier study on enzymes in the human nephron (14), a morphologically normal kidney biopsy (F) was taken as normal tissue. As shown in table 1, alkaline phosphatase activity in proximal tubules is about 10 times that of distal nephron segments in accordance with the high brush-border membrane content in proximal regions (18). No difference was found between the convoluted and straight part of cortical proximal tubules. In contrast, N-acetyl-β-*D*-glucosaminidase activity appears to be distributed inhomogeneously along proximal tubules with higher activities in the straight portion (tab. 2). Interestingly, in this segment, N-acetyl-β-*D*-glucosaminidase activities were found to differ strongly among biopsies E and F. In glomeruli and distal nephron segments, N-acetyl-β-*D*-glucosaminidase activity is relatively high compared with alkaline phosphatase activity.

Biopsies taken during transplantation operations (A_1, B_1, C_1, D_1) displayed different activities of both enzymes in proximal tubules (tabs. 1–4). In biopsy D_1, alkaline phosphatase activities were reduced to about 50% irrespective of post-transplantation kidney function (tab. 1), whereas the proximal convoluted tubules of biopsies A_1, B_1, and C_1 as well as the proximal straight tubules of biopsies A_1 and B_1 contained activities similar to those in normal tissues E and F (tab. 3). In functioning allograft D, alkaline phosphatase activities in proximal convoluted and straight tubules

Tab. 1. Specific catalytic activities of alkaline phosphatase in single cortical nephron segments of human kidneys (4-methylumbelliferone, μmol · min^{-1} · g^{-1} dry weight). Values are given as the mean ± standard deviation with the number of determinations in parentheses. The indices represent statistically significant differences: (a) = $p < 0.05$, (b) = $p < 0.02$, (c) = $p < 0.001$, (d) = $p < 0.0001$. Biopsy D_1 was compared with biopsies E and F(*), biopsies D_2 and D_3 were compared with biopsy D_1.
Abbreviations:
G = glomerulus,
PCT = proximal convoluted tubule,
cPST = cortical proximal straight tubule,
cTAL = cortical thick ascending limb of *Henle*'s loop,
DCT = distal convoluted tubule,
cCD = cortical collecting duct.

Segment	Kidney E (normal part of a kidney with hypernephroma)	Kidney F (microscopic haematuria, morphologically normal)	Kidney allograft D (functioning)		
			Biopsy D_1 (intraoperative)	Biopsy D_2 (normuria, 7 days after transplantation)	Biopsy D_3 (normuria, 20 days after transplantation)
G	0.5 ± 0.2 (10)	1.2 ± 0.8 (6)	1.2 ± 0.7 (5)	2.2 ± 0.8 (7) (a)	1.2 ± 0.4 (8)
PCT	25.9 ± 13.9 (49)	28.2 ± 10.1 (33)	14.9 ± 7.4 (31) (d, d*)	35.8 ± 13.5 (20) (d)	25.4 ± 13.9 (31) (c)
cPST	23.4 ± 8.5 (33)	25.0 ± 8.4 (20)	12.2 ± 6.1 (25) (d, d*)	26.2 ± 12.8 (21) (d)	26.3 ± 15.0 (16) (c)
cTAL	3.2 ± 1.4 (6)	3.2 ± 1.2 (10)	2.4 ± 0.9 (6)	1.6 ± 0.7 (6)	3.6 ± 1.8 (6)
DCT	3.7 ± 1.7 (7)	2.6 ± 1.9 (7)	1.9 ± 0.6 (4) (a)	2.6 ± 1.6 (8)	3.6 ± 1.3 (8) (b)
cCD	4.1 ± 1.4 (5)	–	–	1.5 ± 0.8 (4)	3.0 ± 1.3 (4)

Tab. 2. Specific catalytic activities of N-acetyl-β-*D*-glucosaminidase in single cortical nephron segments of human kidneys (4-methylumbelliferone, μmol · min^{-1} · g^{-1} dry weight). Values are given as the mean ± standard deviation with the number of determinations in parentheses. The indices represent statistically significant differences: (a) = $p < 0.02$, (b) = $p < 0.01$, (c) = $p < 0.002$, (d) = $p < 0.001$, (e) = $p < 0.0001$. Biopsy F was compared with biopsy E, biopsy D_1 with biopsies E and F(*), and biopsies D_2 and D_3 were compared with biopsy D_1.
The same abbreviations for nephron segments as in table 1 were used.

Segment	Kidney E (normal part of a kidney with hypernephroma)	Kidney F (microscopic haematuria, morphologically normal)	Kidney allograft D (functioning)		
			Biopsy D_1 (intraoperative)	Biopsy D_2 (normuria, 7 days after transplantation)	Biopsy D_3 (normuria, 20 days after transplantation)
G	5.4 ± 0.9 (17)	4.2 ± 0.8 (4) (a)	6.3 ± 0.7 (10) (b, d*)	7.0 ± 0.8 (8)	4.9 ± 0.9 (10) (c)
PCT	33.8 ± 26.3 (81)	27.3 ± 19.6 (18)	17.5 ± 12.4 (30) (e)	18.6 ± 5.3 (26)	22.3 ± 9.9 (42)
cPST	77.0 ± 41.0 (31)	41.6 ± 16.2 (17) (d)	89.2 ± 48.6 (26) (e*)	57.4 ± 32.6 (29) (b)	39.0 ± 18.1 (22) (e)
cTAL	15.3 ± 7.3 (15)	15.6 ± 4.5 (10)	8.5 ± 6.1 (12) (a, b*)	12.3 ± 4.5 (9)	7.7 ± 4.6 (15)
DCT	13.7 ± 5.5 (17)	8.4 ± 3.7 (8) (b)	10.1 ± 4.5 (13)	11.9 ± 2.0 (11)	13.0 ± 4.7 (14)
cCD	10.7 ± 5.4 (5)	–	–	18.2	13.0 ± 4.6 (4)

had reached the levels of biopsies E and F at the 7th postoperative day (D_2) (tab. 1). In contrast to this, N-acetyl-β-*D*-glucosaminidase activity was found to be uniformly reduced to about 50% in proximal convoluted tubules of intraoperative biopsies A_1, B_1, C_1, and D_1, when compared with biopsies E and F (tabs. 2 and 4). This level appears to be maintained in proximal convoluted tubules of functioning allograft D, 7 and 20 days after transplantation (D_2, D_3) (tab. 2). On the other hand, in proximal straight tubules, the enzyme activities of intraoperative biopsies A_1 and C_1 were in the same range as those in kidney F (tab. 4). The activity in allograft D intraoperatively resembled that of biopsy E, but developed into that of biopsy F after 7 and 20 days of post-transplantation function (D_2, D_3) (tab. 2). In proximal straight tubules of biopsy B_1 only half this activity was found (tab. 4).

Biopsies in acute post-transplant renal failure

In the maintenance and recovery phases of acute post-transplant renal failure, gradual activity changes of alkaline phosphatase were detected after 9 days (A_2) and 14 days (B_2) of anuria (tab. 3). Biopsy A_2 displayed the activity of normal renal tissues E and F in proximal convoluted tubules, but slightly reduced activity (61%, $p < 0.01$) in proximal straight tubules, when compared with intraoperative values. In biopsy B_2, alkaline phosphatase activity was decreased in both proximal convoluted (70%, $p < 0.002$) and straight tubules (55%, $p < 0.001$). This activity showed a marked decrease in allograft C recovering from anuria (C_2), with 25% activity ($p < 0.0001$) in proximal convoluted tubules and 60% activity ($p < 0.002$) in proximal straight tubules, corresponding to 35% of the mean value of biopsies E, F, A_1, B_1,

Tab. 3. Specific catalytic activities of alkaline phosphatase in single cortical nephron segments of human kidney allografts with acute post-transplant renal failure (4-methylumbelliferone, $\mu mol \cdot min^{-1} \cdot g^{-1}$ dry weight). Values are given as the mean ± standard deviation with the number of determinations in parentheses. The indices represent the following statistically significant differences: (a) = $p < 0.05$, (b) = $p < 0.01$, (c) = $p < 0.002$, (d) = $p < 0.001$, (e) = $p < 0.0001$. The data of postoperative biopsies were compared with those of corresponding intraoperative ones.
The same abbreviations for nephron segments as in table 1 were used.

Segment	Kidney allograft A		Kidney allograft B		Kidney allograft C	
	Biopsy A_1 (intraoperative)	Biopsy A_2 (post-transplantation anuria for 9 days)	Biopsy B_1 (intraoperative)	Biopsy B_2 (post-transplantation anuria for 14 days)	Biopsy C_1 (intraoperative)	Biopsy C_2 (post-transplantation anuria for 12 days, oliguria for 1 day, polyuria and normuria for 11 days)
G	0.6 ± 0.4 (5)	0.6 ± 0.3 (5)	0.6 ± 0.4 (7)	0.4 ± 0.2 (5)	1.6 ± 0.5 (5)	0.9 ± 0.5 (7) (a)
PCT	31.1 ± 11.5 (20)	36.4 ± 13.6 (23)	29.0 ± 11.0 (32)	20.3 ± 10.3 (33) (c)	31.0 ± 13.8 (20)	7.7 ± 5.2 (40) (e)
cPST	28.3 ± 14.1 (20)	17.3 ± 8.7 (31) (b)	28.5 ± 12.5 (26)	15.8 ± 8.3 (21) (d)	15.2 ± 7.2 (26)	9.1 ± 6.3 (27) (c)
cTAL	2.2 ± 1.1 (6)	1.9 ± 1.3 (8)	3.3 ± 1.3 (7)	3.6 ± 1.9 (10)	2.6 ± 1.2 (9)	2.8 ± 2.1 (8)
DCT	3.9 ± 1.1 (6)	1.0 ± 0.6 (7) (d)	3.1 ± 2.1 (7)	2.6 ± 1.6 (8)	1.8 ± 1.1 (5)	2.2 ± 1.4 (4)
cCD	–	2.5	1.3	1.0 ± 0.5 (2)	–	1.0

Tab. 4. Specific catalytic activities of N-acetyl-β-*D*-glucosaminidase in single cortical nephron segments of human kidney allografts with acute post-transplant renal failure (4-methylumbelliferone, $\mu mol \cdot min^{-1} \cdot g^{-1}$ dry weight). Values are given as the mean ± standard deviation with the number of determinations in parentheses. The indices represent the following statistically significant differences: (a) = $p < 0.05$, (b) = $p < 0.01$, (c) = $p < 0.002$, (d) = $p < 0.001$, (e) = $p < 0.0001$. The data of postoperative biopsies were compared with those of corresponding intraoperative ones.
The same abbreviations for nephron segments as in table 1 were used.

Segment	Kidney allograft A		Kidney allograft B		Kidney allograft C	
	Biopsy A_1 (intraoperative)	Biopsy A_2 (post-transplantation anuria for 9 days)	Biopsy B_1 (intraoperative)	Biopsy B_2 (post-transplantation anuria for 14 days)	Biopsy C_1 (intraoperative)	Biopsy C_2 (post-transplantation anuria for 12 days, oliguria for 1 day, polyuria and normuria for 11 days)
G	7.0 ± 1.0 (4)	10.6 ± 2.0 (10) (d)	7.9 ± 0.7 (6)	11.7 ± 1.1 (10) (e)	5.8 ± 0.5 (6)	10.4 ± 1.5 (9) (e)
PCT	15.3 ± 6.2 (16)	36.7 ± 15.1 (23) (e)	11.4 ± 8.6 (39)	59.4 ± 29.8 (32) (e)	14.5 ± 10.5 (28)	41.7 ± 25.1 (34) (e)
cPST	49.4 ± 13.1 (19)	58.2 ± 20.2 (16)	23.5 ± 16.3 (28)	136.8 ± 71.2 (27) (e)	43.9 ± 19.2 (21)	82.6 ± 39.6 (20) (d)
cTAL	11.3 ± 4.3 (13)	21.6 ± 5.4 (17) (e)	9.4 ± 4.7 (16)	27.9 ± 9.1 (14) (e)	12.8 ± 5.8 (15)	18.1 ± 8.3 (16) (a)
DCT	11.7 ± 4.3 (7)	22.1 ± 6.1 (9) (c)	8.3 ± 3.8 (20)	29.5 ± 9.5 (11) (e)	10.0 ± 4.2 (14)	16.3 ± 7.7 (17) (b)
cCD	7.3	17.4 ± 8.3 (3)	3.2 ± 0.9 (2)	28.5 ± 8.9 (5) (c)	13.1 ± 3.7 (2)	11.7

D_2, and D_3. N-acetyl-β-*D*-glucosaminidase activity showed a very different behaviour in postoperative biopsies A_2, B_2, and C_2 (tab. 4). In all cortical nephron segments, activities were increased, when compared with the respective intraoperative values: in glomeruli to 148–179%, in thick ascending limbs of *Henle*'s loop to 141–297%, and in distal convoluted tubules to 163–355%. The highest activities in proximal tubules were measured in biopsy B_2: 521% ($p < 0.0001$) in the convoluted and 582% ($p < 0.0001$) in the straight part. These high activities exceed that of normal renal tissue two- to three-fold. In proximal convoluted tubules of biopsies A_2 and C_2, N-acetyl-β-*D*-glucosaminidase activities had returned to the level of normal renal tissue (E, F), corresponding to 240% ($p < 0.0001$) and 288% ($p < 0.0001$), respectively, of intraoperative values. Whereas the activity of proximal straight tubules in biopsy A_2 remained approximately constant, in biopsy C_2 it amounted to 188% ($p < 0.001$) of the intraoperative value.

Biopsy in acute rejection

In the acute rejection episode of allograft D (D_4), 25 days after transplantation, alkaline phosphatase activity was drastically decreased in proximal convoluted and straight tubules to 24% ($p < 0.0001$) and 21% ($p < 0.0001$) of the values in biopsy D_3 (tab. 5). In addition, glomeruli and distal tubules displayed reduced enzyme activities. The changes were found to be very heterogeneous according to the histological appearance of the tissue. Figure 1 demonstrates this heterogeneity with respect to round-cell infiltration and succinate dehydrogenase activity, as also described by *Grégoire & Gepts* (13). Since microdissection was done in sections adjacent to the stained sections, isolated specimens could be correlated to normal or reduced succinate dehydrogenase activity (19). This classification permits discrimination between proximal tubules with alkaline phosphatase activities nearly as low as those in distal tubules, corresponding to tissue blanks of normal proximal tubules, and proximal tubules with significantly higher activities (tab. 5). In contrast, N-acetyl-β-*D*-glucosaminidase activity showed mainly an additional decrease to 59% ($p < 0.0001$) in proximal convoluted tubules, when compared with biopsy D_3, but was slightly increased (136%, $p < 0.02$) in proximal straight tubules. Distally, there were only moderate activity changes. The changes of N-acetyl-β-*D*-glucosaminidase activity were independent of round-cell infiltration density and succinate dehydrogenase activity, except in distal convoluted tubules (tab. 6).

Discussion

The present study reports on biochemical characterization of cellular compartments in single nephron segments of human kidney allografts with impaired function. Alterations in brush-border membranes and lysosomes were quantified by means of the marker enzymes alkaline phosphatase and N-acetyl-β-*D*-glucosaminidase, respectively. Since these enzymes are known to be excreted into urine (1, 3, 20), their renal activity may be balanced by enzymuria and enzyme regeneration.

Catalytic activities of alkaline phosphatase along the human nephron were considered to be normal in biopsies E and F as well as in biopsies D_2 and D_3 with post-transplantation function. In these biopsies, N-acetyl-β-*D*-glucosaminidase activities in glomeruli and distal nephron segments are obviously normal.

Tab. 5. Specific catalytic activities of alkaline phosphatase in single cortical nephron segments of a human kidney allograft with acute rejection (4-methylumbelliferone, μmol · min^{-1} · g^{-1} dry weight). The values of the first column were classified into two groups according to the appearance of tubular staining for succinate dehydrogenase activity (fig. 1). The data represent the mean ± standard deviation with the number of determinations in parentheses and with the following indices for statistically significant differences: (a) = $p < 0.05$, (b) = $p < 0.0001$. The values of biopsy D_4 (first column) were compared with those of biopsy D_3 (tab. 1). In addition, when classified, the values of the second column were compared with those of the third column.
The same abbreviations for nephron segments as in table 1 were used.

Segment	Kidney allograft D		
	Biopsy D_4 (acute rejection, 25 days after transplantation)	Biopsy D_4 (segments with normal succinate dehydrogenase activity)	Biopsy D_4 (segments with reduced succinate dehydrogenase activity)
G	0.8 ± 0.3 (8) (a)	–	–
PCT	6.0 ± 4.3 (31) (b)	7.9 ± 3.5 (17)	2.8 ± 1.4 (12) (b)
cPST	5.4 ± 4.0 (24) (b)	8.1 ± 2.3 (10)	2.8 ± 1.4 (11) (b)
cTAL	1.5 ± 1.0 (7) (a)	1.8 ± 1.2 (3)	1.4 ± 0.9 (4)
DCT	0.3 ± 0.2 (5) (b)	–	–

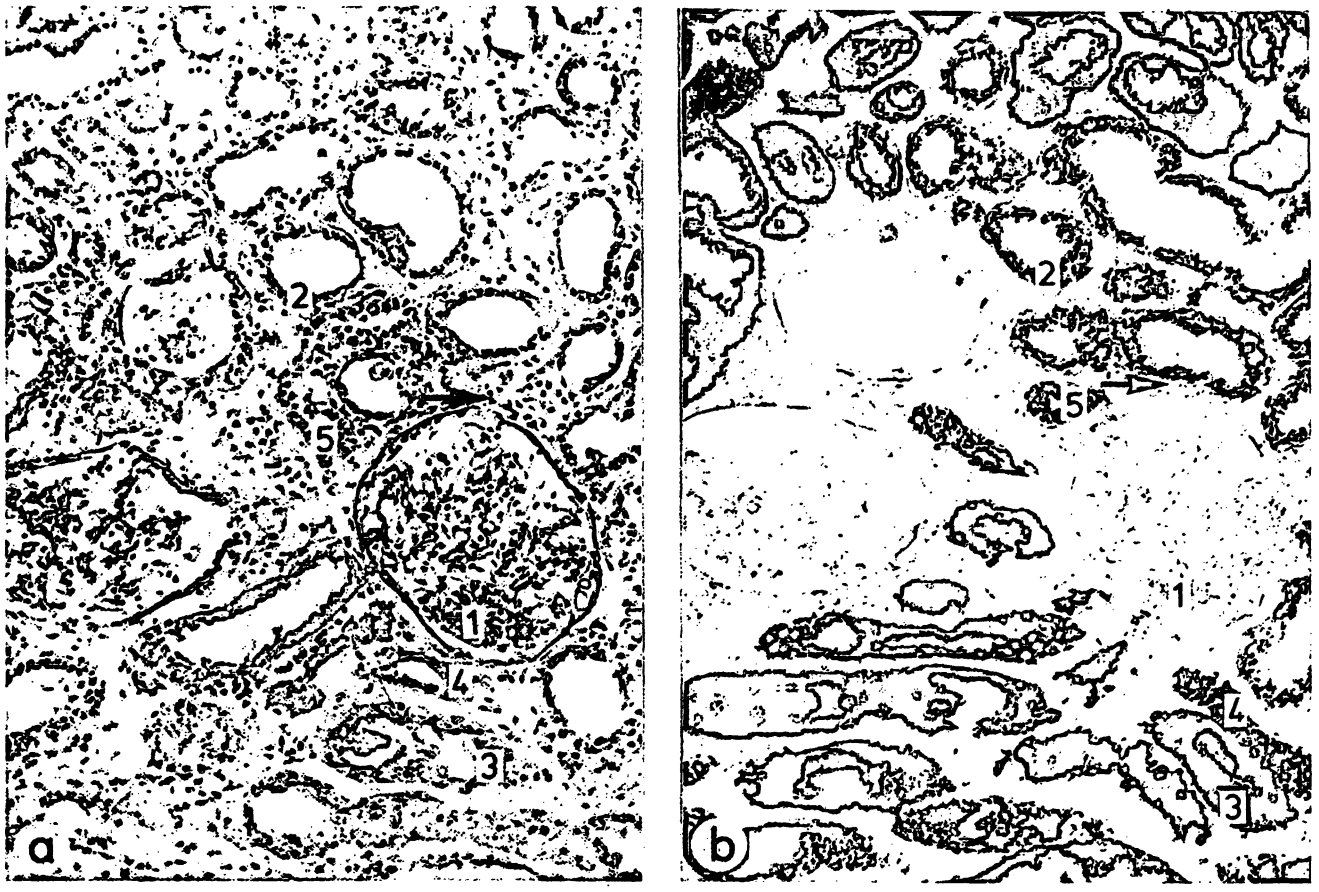

Fig. 1. Cryostat sections (16 µm thick) of kidney allograft D with acute rejection (biopsy D_4). Magnification × 113.
a) First of three consecutive sections stained with periodic acid/*Schiff*'s base reagent with inhomogeneously distributed round-cell infiltration (→) and with increased interstitial spaces.
b) Third section of this series stained for succinate dehydrogenase activity. This activity appears to be reduced in areas with increased round-cell infiltration (→) irrespective of the nephron region (19).
1 = glomerulus,
2 = proximal convoluted tubule,
3 = cortical proximal straight tubule,
4 = cortical thick ascending limb of *Henle*'s loop,
5 = distal convoluted tubule.

Tab. 6. Specific catalytic activities of N-acetyl-β-*D*-glucosaminidase in single cortical nephron segments of a human kidney allograft with acute rejection (4-methylumbelliferone, µmol · min^{-1} · g^{-1} dry weight). The values of the first column were classified into two groups to give the second and the third column, as described in table 5. The data represent the mean ± standard deviation with the number of determinations in parentheses and with the following indices for statistically significant differences: (a) = $p < 0.02$, (b) = $p < 0.002$, (c) = $p < 0.0001$. As in table 5, the values of biopsy D_4 were compared with those of biopsy D_3 (tab. 2), and those of the second and the third column were compared with each other.
The same abbreviations for nephron segments as in table 1 were used.

Segment	Kidney allograft D		
	Biopsy D_4 (acute rejection, 25 days after transplantation)	Biopsy D_4 (segments with normal succinate dehydrogenase activity)	Biopsy D_4 (segments with reduced succinate dehydrogenase activity)
G	4.6 ± 0.8 (9)	–	–
PCT	13.2 ± 5.1 (24) (c)	11.6 ± 3.7 (5)	13.6 ± 5.4 (19)
cPST	53.2 ± 17.3 (20) (a)	56.9 ± 16.3 (12)	47.6 ± 19.8 (7)
cTAL	4.2 ± 2.5 (20) (a)	3.2 ± 1.6 (10)	5.3 ± 3.0 (9)
DCT	16.6 ± 10.4 (32)	9.7 ± 7.2 (12)	21.0 ± 10.2 (19) (b)
cCD	5.6 ± 3.4 (3)	–	–

However, it is more difficult to define normal values in proximal tubules. In proximal convoluted tubules of biopsies E and F, they are thought to be normal. In proximal straight tubules of biopsy F, the activity was in the range of that in the same segments of transplant biopsies D_2 and D_3, and it was therefore assumed to be normal, confirmed by the respective values obtained from intraoperative transplant biopsies A_1 and C_1. Apparently, immunosuppressants had not influenced both enzyme activities. Both enzymes represent cellular parameters which appear to be highly variable in proximal tubules, as seen in the functioning allograft with consecutive biopsies D_1, D_2, and D_3. Such variations may be due to the anamnesis of the kidneys. In addition, the age of the patients has to be taken into account, as in case E. In old rats, for example, a decrease of alkaline phosphatase activity (21) and focal brush-border loss as well as an increase in the relative volume of autophagic vacuoles (22) have been described.

Characteristically, both enzymes display their highest activities in proximal tubules, in parallel with the cellular content of brush-border membranes and lysosomes, as also demonstrated in rat kidney (18, 23). The values of alkaline phosphatase activity in glomeruli, distal tubules, and collecting ducts are approximately as low as tissue blanks of proximal tubules. In contrast to rat (24) and mouse (4) nephrons, which show the highest alkaline phosphatase activity in proximal convoluted tubules, the activity in human cortical proximal straight tubules is the same as that in the convoluted part. In addition, the distribution pattern of N-acetyl-β-*D*-glucosaminidase in proximal tubules of the human nephron, with highest activity in proximal straight tubules, is the inverse of that in rat and rabbit nephrons (7, 25).

The distribution patterns of both enzymes appear to be altered in biopsies of human kidney allografts taken intraoperatively. Thus, loss of microvillar enzymes as well as intracellular and extracellular release of lysosomal enzymes, both described in kidney cryopreservation (26, 27), do not occur uniformly. However, the outcome of the transplantation does not seem to depend on intact brush-border membranes, as suggested from alkaline phosphatase activities in proximal tubules of intraoperative biopsies A_1, B_1, and D_1. Favoured by good metabolic conditions, brush-border membranes can be rapidly restored (28, 29), as indicated by normal alkaline phosphatase activities in biopsies D_2 and D_3, 7 and 20 days after transplantation. N-Acetyl-β-*D*-glucosaminidase release may lead to reduction of enzyme activity to nearly 50% of the normal level in proximal convoluted tubules of the intraoperative biopsies, irrespective of post-transplantation function. This level is maintained in functioning allograft D (D_2, D_3), corresponding to the well-known increased enzymuria of transplanted kidneys (3, 30). The low activities of N-acetyl-β-*D*-glucosaminidase in proximal convoluted tubules of biopsies A_1, B_1, and C_1 are only slightly above those of distal tubules. On the basis of the isoenzyme profile in rabbit nephron, compared with that of human urine (30), *Bourbouze* et al. (25) also considered proximal convoluted tubules as the source of urinary N-acetyl-β-*D*-glucosaminidase. Interestingly, in intraoperative biopsy of allograft B (B_1) with severest functional and cellular alterations (14), the proximal straight tubules also showed a decreased N-acetyl-β-*D*-glucosaminidase activity. This may indicate that tubular cells are more severely affected by preceding warm and cold ischaemia. This lysosomal enzyme release of cryopreserved and transplanted kidneys may be due to ischaemia- and reperfusion-induced fragility of tubular lysosomes (31).

In the maintenance and recovery phases of acute post-transplant renal failure, renal alkaline phosphatase and N-acetyl-β-*D*-glucosaminidase activities diverge considerably. The finding of relatively high alkaline phosphatase activities in proximal tubules of anuric allografts (A_2, B_2) corresponds to the observation of *Olsen* et al. (12) that the brush-border profile showed only weak correlation with renal function in human kidney biopsies. The preferential activity reduction in proximal straight tubules of both biopsies is consistent with the morphological observations of *Venkatachalam* et al. (32), who showed that proximal straight tubules display greater vulnerability than proximal convoluted tubules in the early phase of experimentally induced mild renal ischaemia of the rat. The present finding suggests that in the advanced stage of human acute renal failure, the regeneration rate of brush-border membranes may be higher in the convoluted part than in the straight part of proximal tubules. Regeneration processes do occur in biopsies taken in anuria (A_2, B_2) and in the recovery phase from anuria (C_2), as indicated by marked postoperative activity increases of N-acetyl-β-*D*-glucosaminidase along the whole nephron, again with the greatest effect in biopsy B_2. This enhanced catabolic capacity may parallel the highly active autophagy that is implicated in cellular recovery (31). In biopsies A_2 and B_2, due to anuria, the renal N-acetyl-β-*D*-glucosaminidase content is determined by enzyme synthesis and by the degradation. Additionally, in biopsy C_2, the overshoot of this enzyme activity is thought to be counteracted by enzymuria out of proximal convoluted tubules, as known from functioning allograft D

(D_2, D_3). *Cantin* et al. (33) observed that 10 days after experimentally induced ischaemia, a similar increase in lysosomal enzyme activities in rat kidney cortex homogenates was not associated with a considerably altered morphological appearance of lysosomes in tubular cells.

In acute rejection crisis of allograft D, biopsy D_4 was taken in the early phase of dysfunction. The drastic reduction of alkaline phosphatase activities in proximal convoluted and straight tubules, with a slight decrease of N-acetyl-β-*D*-glucosaminidase activity in proximal convoluted tubules, when compared with biopsy D_3 in good function, indicate that the activities are determined mainly by enzymuria. This is substantiated by the highly significant difference of alkaline phosphatase activities between areas with normal and with reduced succinate dehydrogenase activities without any sign of regeneration of brush-border membranes. Comparably low alkaline phosphatase activities were found in proximal convoluted and straight tubules of biopsy C_2 in the recovery phase from anuria, with the clinical symptoms of acute rejection beginning 2 days after the puncture. Therefore, the low enzyme activities may be attributed to enzymuria just around the time of biopsy. Thus, after a stimulus, microvillar alkaline phosphatase seems to be shed (20) and excreted in peaks followed by regeneration periods, as described by *Butterworth* et al. (34) in human acute renal failure. On the other hand, N-acetyl-β-*D*-glucosaminidase release into urine with subsequent enzyme regeneration in tubular cells seems to occur rather continuously, as seen in biopsies D_2 and D_3. In biopsy D_4, the rejection episode leads to an additional slight activity decrease in proximal convoluted tubules. This is consistent with the morphometrical findings of *Pfaller* (18) and the biochemical ones of *Venkatachalam* et al. (28), who observed no significant lysosomal alterations, but marked brush-border loss in the early phase of experimentally induced renal ischaemia of the rat.

In addition to enzymuria and enzyme regeneration, it has to be taken into account that specific adaptive increases of N-acetyl-β-*D*-glucosaminidase activity occur in renal tissue and may explain the high activities of this enzyme in proximal straight tubules of biopsies E and D_1. *Le Hir* et al. (35) suggested that such activity increases are associated with enlarged reabsorption of glycoproteins from the ultrafiltrate. While the stimulus for the present high N-acetyl-β-*D*-glucosaminidase activities is unknown, the well-known proteinuria of allograft C may be an additional cause for the high enzyme activity of proximal tubules in biopsy C_2, particularly in the straight portion.

It has to be mentioned that the variety of activity changes of both enzymes may be associated with changes of tissue dry weight used as reference unit. However, a decrease of cellular mass by brush-border loss leading to morphologically detectable flattened proximal tubular cells (11, 32) has not yet been quantified. In addition, the changes of catalytic activities of alkaline phosphatase and N-acetyl-β-*D*-glucosaminidase were measured as total enzyme activities: Quantification of the isoenzymic pattern of both enzymes (25, 36, 37) was not possible at the nanogram level of tissue specimens. Furthermore, the use of lyophilized tissue samples does not permit to determine the degree of lysosomal integrity which is known to be decreased under pathological conditions (31).

In conclusion, the present results demonstrate that renal activities of the brush-border enzyme alkaline phosphatase and the lysosomal enzyme N-acetyl-β-*D*-glucosaminidase undergo various individual changes in renal dysfunction which are dependent on intranephronal location and time. While proximal straight tubules appear to be more sensitive with respect to loss of alkaline phosphatase activity, released N-acetyl-β-*D*-glucosaminidase originates from proximal convoluted tubules. The unspecific overshoot of activity of the latter enzyme in the maintenance and recovery phases of acute post-transplant renal failure, detected in the whole nephron, is considered to be reactive to various cellular alterations following transplantation. These alterations include markedly decreased areas of basolateral and inner mitochondrial membranes in distal tubules (14) as well as distorted brush-border membranes in proximal tubules. This complexity of cellular reactivity to injury implies that it is difficult to interpret enzymuria with respect to the nature and degree of renal damage.

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